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Reproductive partitioning in communally breeding guira cuckoos, *Guira guira*

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Abstract Guira cuckoos, *Guira guira*, exhibit a rare polygynandrous reproductive system with groups containing several male and female breeders, allowing for important tests of reproductive skew models. Female reproductive strategies involve leaving the group, varying clutch size, egg ejection and infanticide, among others. Here we examined the predictions of reproductive skew models relative to reproductive partitioning among females in groups. We used yolk protein electrophoresis to identify individual females' eggs in joint nests. We found that reproductive partitioning favors early-laying females, which lay and incubate more eggs than females that begin laying later. Because the female that lays first tends to switch between repeated nesting bouts, and females do not always contribute eggs to each bout, female reproductive success tends to equalize within groups over time. The pattern of reproductive partitioning differs from that described for anis, another crotophagine joint-nester. We calculated reproductive skew indices for groups in 2 years, for both laying and incubation, as well

as an overall population value. These were compared to random skew generated by simulations. Varying degrees of skew were found for different groups, and also across sequential nesting bouts of the same groups. Overall, however, skew did not deviate from random within the population. Nests that reached incubation tended to have lower skew values during the laying phase than nests terminated due to ejection of all eggs followed by desertion. Groups had higher reproductive skew indices in their first nesting bout of the season, and these nests frequently failed. These results illustrate the importance of social organization in determining not only individual, but group success in reproduction, and highlight the flexibility of vertebrate social behavior.

Keywords Communal breeding · Guira cuckoo · *Guira guira* · Reproductive skew · Yolk protein electrophoresis

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Introduction

Hamilton's rule and inclusive fitness model (Hamilton 1964) have served as guiding principles to understand the evolution of sociality. Since the 1970s there has been an explosion in the number of published studies on the evolution of reproductive sharing in communally breeding birds, mammals and insects (Woolfenden and Fitzpatrick 1984; Brown 1987; Koenig and Mumme 1987; Jamieson 1997). The first models of reproductive sharing appeared over a decade ago (Vehrencamp 1979, 1983a, 1983b; Emlen 1982; Emlen and Vehrencamp 1983) and were supplemented more recently with a burst of new models to explain patterns of reproduction for individuals living in groups (Reeve and Ratnieks 1993; Keller and Reeve 1994; Reeve and Keller 1995; Reeve 1998). These models attempt to predict the degree to which reproduction is biased, or skewed, in favor of dominant individuals. These 'concessions' models assume that dominants can control breeding opportunities within the group, and that the presence of subordinate helpers increases the fitness of dominants. Dominants offer 'staying incentives'

to subordinates by allowing them some degree of reproduction, thus dissuading them from either leaving the group or engaging in fights to assume control. The option of leaving is more attractive for subordinates if there are chances of successful dispersal and breeding, and if they are unrelated to the dominant's offspring, and thus unable to accumulate indirect fitness benefits. Thus, these models predict high skew when group members are closely related, when ecological constraints reduce the chances of dispersal and independent breeding by subordinates, and when group living yields considerable advantages by increasing overall productivity. Some studies have supported the predictions generated by 'concessions' models (e.g. Creel and Waser 1991; Reeve 1991; Bourke and Heinze 1994; Jamieson 1997).

However, observations of obvious conflict within groups and evidence of subordinate reproduction (Clutton-Brock 1998) have led to the development of alternative models that may or may not assume control by dominants. In these, some important and previously neglected issues were addressed, including the partitioning of reproduction among subordinates in multi-member groups, the effects of saturated group sizes, skew through infanticide, and incomplete reproductive control by dominants (Reeve and Ratnieks 1993; Cant 1998; Clutton-Brock 1998; Reeve et al. 1998; Johnstone and Cant 1999a, 1999b; Johnstone et al. 1999; Reeve and Emlen 2000).

The 'incomplete control model' suggests that dominants are incapable of maintaining control of reproduction as the fighting and competitive abilities of individuals become more equal within the group (Reeve and Ratnieks 1993; Cant 1998; Clutton-Brock 1998; Reeve et al. 1998). Reproduction of subordinates in some societies has also been explained in a recent 'beneficial sharing model' that incorporates the cost of producing young (Cant and Johnstone 1999). The model assumes control of reproduction by the dominant female, but when the cost of producing additional young is sufficiently high, the dominant will benefit by sharing reproduction with subordinates. When the cost of producing young is relatively low, the model predicts a positive correlation between skew and relatedness, as predicted by previous 'concessions' models. When costs are high and accelerating (e.g. communally-breeding birds and mammals), the model predicts no association or a negative correlation between skew and relatedness. Thus, while still assuming that dominants control reproductive sharing within the group, this model presents more flexible predictions from those of traditional 'concessions' models, as it takes into consideration the reproductive variability exhibited by different taxa.

Most recent work testing the various reproductive skew models has been done on social insects (Bourke and Heinze 1994; Heinze 1995; Reeve and Keller 1995), and data on the reproductive skew of communally breeding vertebrates are still too scarce to evaluate the merits or generality of skew models (Johnstone and Cant 1999a).

Social vertebrates are especially important to test these models because of their social plasticity (Jamieson 1997).

This study attempts to assess reproductive skew patterns in a South American, communally breeding bird with a complex and variable social structure. Guira cuckoos (*Guira guira*) live and reproduce in groups that may have upwards of 15 members, and only rarely nest in pairs. Membership in groups is retained over at least 4 years (R.H. Macedo, personal observation, 2002), and probably beyond. A long-term study of a population in central Brazil revealed that these birds are polygynandrous joint nesters, with communal clutches of as many as 26 eggs (Macedo 1992; Quinn et al. 1994). Reproductive conflict in this species is evident: 72% of communal clutches ($n=176$) monitored to date exhibited egg ejection (mean communal clutch=8.01 eggs and mean ejected eggs=4.30). Once chicks hatch broods are usually further reduced (80% of 75 broods monitored to date), with at least 50% of this reduction probably due to infanticide (Macedo and Melo 1999).

In a genetic study using multilocus DNA fingerprinting of a limited sample of four breeding groups, it was found that kinship among co-breeders is generally low and quite variable among groups (Quinn et al. 1994). Of 36 adults in the groups, 21 (58%) were sampled, and of the possible 46 pairwise combinations of adults within the groups, including two-sex pairs, only six pairs (13%) had high enough similarity coefficient scores (D) to be considered closely related. Moreover, a study on ecological constraints in this population suggested that the habitat is not only unsaturated, but that empty sites are of high quality and would be available for nesting, if individuals were to leave their groups (Macedo and Bianchi 1997).

Guira cuckoos and anis (subfamily Crotophaginae, Cuculidae) are taxonomically closely related and also exhibit striking similarity in their reproductive behaviors, which include joint nesting and egg ejection or burying behavior (Vehrencamp 1977; Loflin 1983; Macedo 1992). In her pioneering work on communal breeding in anis *Crotophaga sulcirostris*, Vehrencamp (1977) suggested that these birds provide an excellent example of a fairly egalitarian society, where subordinates are allowed some reproduction. However, despite some subordinate reproductive success, Vehrencamp (1977) proposed the existence of a definitive female hierarchy within the group, leading to significant differences in the number of eggs each female retains in the nest.

Here we assess the guira cuckoo system in light of the predictions generated by the 'concessions' versus the 'beneficial sharing' models. Both assume dominant control of reproduction, but have opposing predictions for a vertebrate breeder with costly reproduction, such as the guira cuckoo. Given the above characteristics of the species—low relatedness among co-breeders and relative lack of ecological constraints—the 'concessions' models predict a low level of reproductive skew among females, whereas the 'beneficial sharing' model predicts the opposite, a high level of skew or no association at all.

We also compare the guira cuckoo reproductive system with the more specific reproductive model proposed by Vehrencamp (1977) for anis. We used a newly developed biochemical method to identify the eggs of individual guira cuckoo females in communal clutches.

Methods

Study site and egg collection

We monitored reproduction of guira cuckoo groups from a population in a semi-urban area of 30 km² (15°47'S, 47°56'W) near Brasilia, Brazil from August to December of 1998 and 1999. We placed nylon fishing nets beneath all old nests that had been used by the birds in previous years, as well as beneath new nests, to collect ejected eggs. After nesting activity was detected, we visited nests daily to check for freshly laid eggs. To estimate group size, we counted the number of adults present around the nest at each visit. The maximum number counted in consecutive visits was used as the estimated group size, and this number tended to remain stable in repeated nesting bouts of the group. We collected 297 eggs from nests (most within 5 h of laying, and some after incubation started), substituting them with dummy eggs. These dummy eggs were numbered sequentially during substitution and monitored to record ejection activity. Visits to the nests ceased when the birds started incubating the clutch of dummy eggs, or until ejection of all eggs, which is common in unmanipulated nests, eventually led to desertion.

Dummy eggs were either: (1) guira cuckoo eggs collected outside the study area; or (2) guinea hen or pigeon eggs that approximated guira cuckoo eggs in size and shape. The non-guira cuckoo dummy eggs were hand-painted with non-toxic acrylic paint to match the blue-green background color of guira cuckoo eggs, while the eggshell markings were replicated using white-out paint.

To detect whether dummy eggs disrupted normal ejection patterns, we compared the percentage of ejected eggs per nest from these nests with those studied during the same period in other years (1987, 1988, 1994), where no dummy eggs were used. We performed a normalizing arcsine transformation (Zar 1999) on this variable. There was no difference in the percentage of tossed eggs among years when no dummy eggs were used (ANOVA, $F_{1,59}=0.029$, $n=61$ nests, $P=0.867$) and between years when dummy eggs were used (Student's t -test, $t=-0.494$, $n=42$ nests, $P=0.62$). Thus, we pooled the data for all years where no dummy eggs were used (1987, 1988 and 1994) for comparison with the pooled data from years where dummy eggs were used (1998 and 1999). There was no difference in the proportion of ejected eggs between years with or without dummy eggs (Student's t -test, $t=-1.118$, $n=103$ nests, $P=0.28$), which indicates that the introduction of dummy eggs did not interfere with the normal process of egg laying and ejection.

The apparent inability of females to distinguish between real versus dummy eggs, at least in size, may stem from the fact that guira cuckoo eggs vary greatly in mass, ranging from approximately 16 to 32 g (unpublished data). Furthermore, in a parallel study (Cariello et al. 2003) assessing the uniformity of egg characteristics for individual females, we found that about 55% of all eggs could not be assigned to the correct mothers based on dimensions and eggshell patterns. Thus, it is unlikely that females can recognize their own eggs reliably and eject others' eggs.

Egg maternity determination

We did not identify which individuals laid which eggs, an impossible task given the height of the nests, difficulty in capturing and banding females, and impossibility of continuous observation of a large sample of nests. Instead, we applied an indirect method to

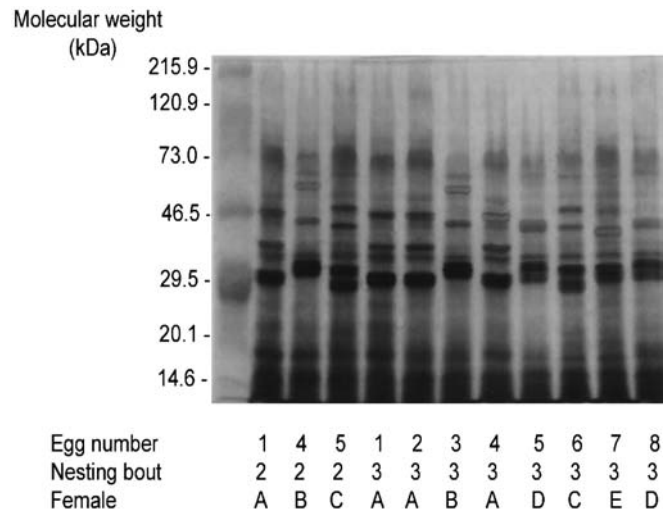


Fig. 1 SDS-PAGE of yolk proteins of eggs from the second and third nesting bouts of guira cuckoo group 1999/Vargem, in which three of six eggs laid were analyzed from the second bout (the remaining three eggs were ejected to the ground), and the whole communal clutch of eight eggs was analyzed from the third bout. In the second bout, eggs 1, 4 and 5 had distinct banding patterns, indicating three females. These were represented in the third nesting bout, with the additional presence of two new females (banding patterns D and E). Egg yolk proteins were digested by trypsin and fragments visualized by silver staining. The migration positions of the molecular weight standards are indicated on the left

determine female egg ownership. The collected eggs, both ejected in the fishing net and from nests, were opened in the laboratory and yolk samples were taken of non-incubated eggs only, which were frozen and later used in a newly-developed protein electrophoresis method that allowed us to determine the maternity of eggs (Cariello et al. 2002). Briefly, yolk proteins in freshly laid eggs are maternally derived, and identical banding patterns are expected in eggs laid by the same female, while different females should present different patterns of yolk proteins.

We ran whole clutches on single SDS-PAGE gels (modified from Laemmli 1970) for comparison of yolk protein polymorphism, and used silver staining for visualizing the proteins. We validated our results by conducting preliminary analyses using known female budgerigar (*Melopsittacus undulatus*) eggs and ovarian egg follicles obtained from known guira cuckoo females. The technique was subsequently applied in the maternal identification of eggs in communal clutches of free-living guira cuckoos in the study population. An example of a gel from a guira cuckoo nest is shown in Fig. 1. Using this technique, we found a mean of 4.81 bands per individual (using only those clearly defined) and, using Andersson and Åhlund's (2001) approach for albumen bands, estimated the probability of two females in a nest sharing the same banding patterns as 6.56×10^{-5} (Cariello et al. 2002).

The female order of entering the laying sequence was determined by identifying the maternity of each egg as it was laid. Thus, the first egg laid in the communal clutch was labeled as laid by female A, the first egg that differed from that egg's pattern as female B, and so on. New eggs with identical protein patterns to previously laid ones were assigned to the same female. In subsequent nesting bouts of the group, eggs were compared to those laid in the first bout and maternal identification was labeled accordingly. When two or more females initiated laying on the same day, they shared the designation of position of entering the laying sequence, and the values for numbers of eggs laid, tossed and incubated were averaged for use in statistical analyses. In the groups monitored, the total number of females contributing eggs to any single nesting bout varied from a solitary female to seven

females. We did not consider single-female nesting bouts in analyses comparing reproductive parameters according to laying position. We also did not use the single nesting bout containing seven females, because the sample was too small to allow comparisons with bouts that had from two to six females.

We were thus able to ascertain the total or minimum number of laying females per nest, the order in which they entered the laying process, the total or minimum number of eggs each female laid and lost through ejection, and the identity of the females represented in the final communal clutch incubated and in successive nesting bouts of groups. The non-incubated eggs for which we were unable to establish maternal identity were usually first-laid eggs in the communal clutch, ejected before the net was in place. But because females seldom lay only a single egg, we are confident that, despite the underestimation of individual clutch size in some instances, this resulted in only a negligible error when determining the representation of females within nests.

In this study we collected the entire egg to answer questions concerning individual investment in eggs, in addition to identifying their maternal origin. However, the extraction of only a small sample of yolk (Schwabl 1993) or albumen (Andersson and Åhlund 2001) through a hole in the eggshell allows the same methodology (protein electrophoresis) and has the added advantage of allowing eggs to hatch.

Determination of reproductive skew indices

Reeve and Ratnieks (1993) and Keller and Vargo (1993) developed an index that quantifies reproductive skew, which varies from 0 (low skew: equitable reproductive partitioning among group members) to 1 (high skew: monopoly of reproduction by a single individual). Another formula for reproductive skew (S) was derived by Pamilo and Crozier (1996): $S = (N_T - Q_E) / (N_T - 1)$, where N_T is the total number of potential breeders and Q_E is the 'effective number' of breeders defined as $Q_E = 1 / \sum p_i^2$, where p_i is the reproductive contribution of the i th female. We used this formula instead of the previous indices because Pamilo and Crozier (1996) argue that the former indices had certain statistical characteristics that led to discontinuities in an otherwise linear relationship. Using this formula, we calculated the reproductive skew for females only, during the egg-laying and incubation phases of each group-year, and averaged (\pm SD) these values to derive estimates when comparing repeated nesting bouts or nests that failed to reach incubation with those that reached incubation.

Potential female breeders included all females identified within the group in consecutive nesting bouts with the yolk protein electrophoresis method. To estimate the total number of potentially breeding females for calculating reproductive skew, we used either: (1) groups that attempted breeding at least twice, giving ample opportunity for all females to breed; or (2) single-attempt breeding groups where at least three females participated in the breeding attempt. Thus, we did not include those single-attempt breeding groups that had less than three females represented in the analyses. This was done to avoid underestimating the number of potentially breeding females in a group. Nonetheless, it is possible that a few females never had any opportunity to breed, and thus were not included in the subgroup of potentially breeding females, yielding underestimated skew values in some instances. We excluded from the analysis one nest where only 33% of the clutch had a maternal identity established. Also, we did not use nests where only a single egg was laid, as reproductive skew can only be considered using whole eggs as the unit of calculation.

To determine whether the observed skew was different from random, we compared observed reproductive skew with that expected if parentage of each egg was randomly assigned to each potentially breeding female in a breeding attempt. Mean random skew was then determined from 1,000 runs of each simulation for each nest and for each breeding attempt. For these calculations, nests with fewer than three eggs were excluded. Sign tests were used to compare observed and random skew both for eggs laid and

for eggs retained. First breeding attempts and any subsequent breeding attempts were considered separately as well as together.

Results

In the 2 study years, we monitored 15 groups that produced 48 nests of which 34 were analyzed in this study due to the availability of information on different aspects of reproduction. In some cases we could not collect data on a nesting bout because the nest was not accessible, although we registered breeding activity for the group. If a nest was found after the first egg was laid, it was not included in analyses that refer to female order of entering laying. We usually knew approximately how many eggs had been ejected prior to our detection of reproductive activity, as ejected eggs were found either in the nets or broken on the ground. This allowed us to determine to what extent the inclusion of such nests in some analyses could compromise the quality of results, and to discard some nests where too much information was lost. It is unlikely that the number of breeding females was underestimated in nests with large communal clutches, even if a few eggs were lost to analysis, as the extended laying period in such nests would have allowed ample opportunity for all females to lay eggs.

We used 195 non-incubated eggs for the PAGE analyses. For 17 of 34 nests (50%), all laid eggs were analyzed by electrophoresis, for eight nests (23.5%), at least 80% of eggs were identified, and for an additional seven nests (20.6%), at least 50% of egg identities were known. For the remaining two nests (5.9%), less than 50% of egg identities were known.

We first analyzed the variability of reproductive parameters (eggs laid, ejected and incubated) for females that had the same position in the order of entering the laying sequence in different groups. For this, we used an ANCOVA that had the number of co-breeding females as the covariate, thus eliminating the variability that could obscure real differences in the dependent variables analyzed (reproductive parameters: eggs laid, ejected or incubated). There was a statistical variation only for number of eggs laid ($F_{5,1} = 3.094$, $n = 33$ females, $P = 0.03$). No statistical variation was found for number of eggs ejected ($F_{5,1} = 0.344$, $n = 30$ females, $P = 0.88$) or eggs incubated ($F_{4,1} = 0.544$, $n = 26$ females, $P = 0.71$). The fact that nesting bouts differed in the number of co-breeding females did not greatly affect the final reproductive output for females in the same position in the laying order.

When considering all females together and notwithstanding the number of co-breeding females in the group, those in the first laying positions laid more eggs relative to those in later positions (Pearson $r = -0.46$, $n = 33$, $P < 0.01$), lost equivalent numbers of eggs due to ejection (Pearson $r = -0.03$, $n = 30$, $P = 0.86$), and ended up with a greater number of eggs incubated in the communal clutch (Pearson $r = -0.40$, $n = 26$, $P = 0.04$; Fig. 2). A direct comparison of the early laying positions (females entering

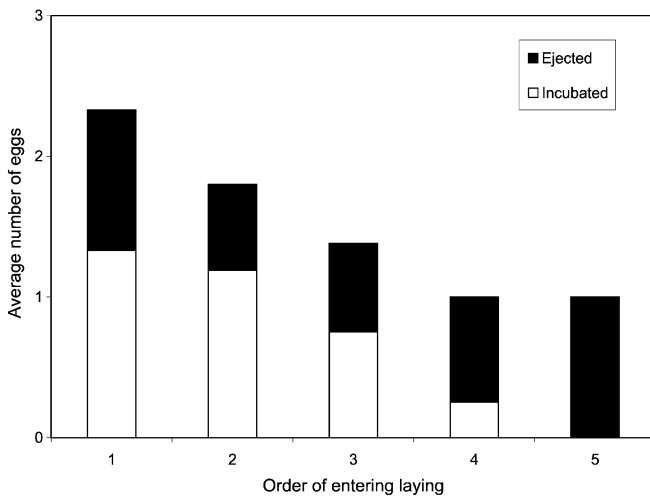


Fig. 2 Mean number of eggs laid, ejected and retained for incubation for females that entered the laying sequence in positions 1 through 5 of nesting bouts

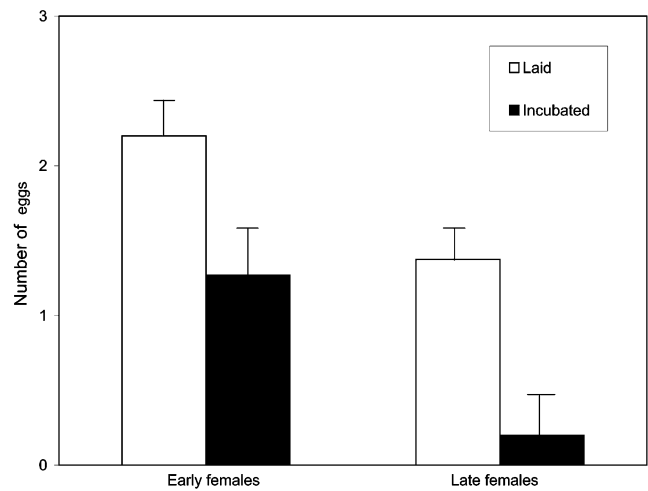


Fig. 3 Mean number of eggs laid and incubated (\pm SE) for females in early (1st and 2nd) vs late (4th through 6th) positions in the group's egg-laying sequence

first and second; $n=20$) with later positions (fourth through sixth; $n=8$) shows that the former laid more eggs than the latter (Mann-Whitney $U=126.00$, $P=0.01$) (Fig. 3). The individual clutch-size difference between early- and late-laying females was of one to two eggs. However, egg ejection equally affected females in early or late positions (Mann-Whitney $U=61.00$, $P=0.73$).

Ten groups, which had from two to ten females, were involved in two to three repeated nesting bouts within seasons, of which we monitored 32 bouts with variable amounts of information on female identity. Despite the loss of information due to occasional eggs being ejected outside the nets, or the fact that some nests were found after laying had started, some interesting patterns emerged. In none of the groups did all females enter all nesting bouts, although some females contributed eggs to all nesting bouts in a season (Table 1). In repeated nesting bouts, different proportions (17–100%) of the groups' females contributed to the communal clutch. The first nesting bout of the season had an average of 38.8% participation of the groups' females ($n=7$ groups). Female participation peaked in the second nesting bout (80.7%, $n=9$ groups), and then declined in the third nesting bout

(70%, $n=6$ groups). The percentage of group females that contributed to the communal clutch was associated with the percentage of eggs that survived ($r=0.47$, $n=19$ bouts, $P=0.05$).

We were able to show that the female order of entering laying changed within groups over the course of the season (Table 1: restricted to data from nests where the identification of most eggs allowed us to determine the order of entering laying for a majority of females). There appears to be no consistent pattern overall, though first-laying females may have occasionally initiated laying in a second or third bout. The first bout was often characterized by the laying of only one female, whose single or few eggs were habitually ejected, and which then usually participated in subsequent nesting bouts. Two or even three females sometimes laid eggs synchronously on the same day. In none of the nests monitored did the last laying female(s) in one bout continue in that position in a subsequent bout.

Two of these groups had three nesting bouts with fairly complete information (76% of all eggs) concerning both egg fate (incubated vs ejected) and maternal identity. We assume that the loss of information concerning the non-

Table 1 Female identity (designated by *capital letters*) in the order of entering the laying sequence in repeated nesting bouts of the same group, followed by the proportion of the groups' females contributing to each nesting bout. *Slashes* cluster new females that entered laying within each day. *Parentheses* cluster females entering laying in an unknown sequence, as a result of nest being found after the onset of laying. *Question marks* designate unidentified females due to eggs being ejected outside nets

Year/Group	Females in group	Female sequence within nests (% female participation)		
		First nest	Second nest	Third nest
1998/Bigode	6	A (17%)	(?, B, C)/A/D/E/F (100%)	B/D, E (50%)
1998/Tchê	10	? /A/B, C (30%)	(C, D, E, F, G, H)/I (70%)	J/C (20%)
1998/Piraúba	2	A (50%)	B (50%)	
1999/Bigode	3	A (33%)	(A, B)/C (100%)	
1999/Tchê	6	(?, A) (17%)	A/B/C/D (67%)	(?, C, E)/F (50%)
1999/Evani	5	?	?/A, B (40%)	(?, B, C, D, E)/A (100%)
1999/Vargem	5	?	A/? , B/C (60%)	(A, B)/C, D/E (100%)
1999/Village	4	A/? (25%)	(?, A, B, C,)/D (100%)	

Table 2 Summary of maternity data for eggs laid in all nesting bouts of groups monitored in 1998–1999. In cases where less than 100% of eggs were analyzed through yolk protein electrophoresis, the number of females indicated represents a minimum estimate of

breeding females. Also shown are observed and randomly assigned reproductive skew indices (*RS*) for those nests that followed analyses inclusion criteria detailed in text (formula for reproductive skew and details on estimation of random skew in text)

Year/group	Bout	Females		Clutch	Laying		Incubation		Nest fate
	No.	In group	In bout	(Proportion analyzed)	Observed RS	Random RS	Observed RS	Random RS	
1998/Alemã	2	5	5	13 (0.87)	0.17	0.27	0.50	0.47	Success
1998/Bigode	1	6	1	1 (1.00)					Fail
	2	6	6	12 (0.63)	0.17	0.33	0.49	0.55	Success
	3	6	3	8 (1.00)	0.62	0.42	0.62	0.43	Success
1998/Cafezal	1	2	1	1 (1.00)					Success
	2	2	2	5 (1.00)	0.08	0.27	0.08	0.27	Success
1998/Danhebert	2		1	1 (1.00)					Fail
1998/Estatuas	2		2	4 (1.00)					Fail
1998/Evani	2	4	4	14 (0.93)	0.13	0.23			Fail
1998/Itajuba	1		1	1 (1.00)					Fail
1998/Pedra Verde	1	6	6	15 (0.73)	0.15	0.37			Fail
1998/Piraúba	1	2	1	2 (1.00)	1.00				Fail
	2	2	1	1 (1.00)					Fail
1998/Quaresmas	1	5	5	11 (0.82)	0.18	0.35	0.58	0.57	Success
1998/Simonato	3	4	4	9 (0.89)	0.16	0.30			Fail
1998/Tchê	1	10	3	7 (0.86)	0.83	0.64			Fail
	2	10	7	16 (1.00)	0.40	0.38	0.49	0.50	Success
	3	10	2	2 (1.00)	0.89				Fail
1999/Belubia	1		2	6 (0.83)					Success
1999/Bigode	1	3	1	2 (1.00)	1.00				Success
	2	3	3	7 (1.00)	0.06	0.29	0.00	0.34	Success
1999/Cafezal	1		2	4 (1.00)					Fail
	2		1	6 (0.33)					Fail
1999/Danhebert	1		2	3 (0.67)					Fail
1999/Estatuas	1		1	2 (0.50)					Fail
1999/Evani	2	5	2	12 (0.83)	0.75	0.33			Success
	3	5	5	12 (0.92)	0.11	0.30	0.75	0.88	Success
1999/Simonato	1	3	3	4 (1.00)	0.17	0.43	0.00	0.50	Success
1999/Tchê	1	6	1	1 (1.00) ^a					Fail
	2	6	4	8 (1.00)	0.56	0.43	0.64	0.55	Success
	3	6	3	12 (0.42)	0.75	0.56			Fail
1999/Vargem	2	5	3	6 (0.50)	0.50	0.66			Fail
	3	5	5	8 (1.00)	0.25	0.38			Fail
1999/Village	1	4	1	7 (0.57)					Fail
	2	4	4	8 (0.63)	0.14	0.44	0.44	0.51	Success

^aNesting bout involved a single intact egg found on the ground (subsequently analyzed through electrophoresis), but the nest was deserted. True size of the clutch is thus uncertain, as other eggs may have been laid much earlier, ejected and consumed by predators on the ground

identified eggs affected all females equally. Thus, these data allowed us to examine the partitioning of reproductive opportunities over a whole season for females with different laying tactics. Sixteen females participated in the six nesting bouts of the two groups. Individual clutch size for these females differed greatly, and ranged from one to six eggs. This difference decreased after egg ejection; in the first group the incubated eggs for females ranged from one to five eggs, while in the second group the range was of zero to three eggs. Most females ($n=11$) laid in one bout only; the remaining females participated in two ($n=4$) and three nesting bouts ($n=1$). Females that

contributed to only one nesting bout in the season laid significantly fewer eggs than did females that partook in two or three nesting bouts (pooled) (Mann-Whitney $U=2.00$, $P<0.01$). These females that participated in only one nesting event also retained fewer eggs in the final incubated clutches (Mann-Whitney $U=6.5$, $P=0.01$).

In Table 2 we summarize the maternity data and show the observed and randomly assigned reproductive skew indices for both laying and incubation phases for groups with single and multiple nesting bouts. First nesting bouts of groups ($n=15$) comprised a single female that monopolized reproduction in approximately half the cases ($n=8$),

but that resulted in nest failure (ejection of eggs and discontinuity of nesting bout) in six (75%) of the nests. In the two remaining cases, either a single egg or two eggs were incubated. In repeated nesting bouts of groups ($n=20$), a single female monopolized the bout in only 15% ($n=3$) of events. Second and third bout clutches met with failure in 50% ($n=10$) of cases.

A comparison of observed reproductive skew levels during laying between first versus later nesting bouts shows that first bouts had a higher skew than later bouts, but the difference is not statistically significant (first bouts, mean= 0.55 ± 0.43 vs later bouts, mean= 0.36 ± 0.28 ; Mann-Whitney $U=67.00$, $n_1=6$, $n_2=16$, $P=0.16$).

Although nests that failed to reach incubation were not statistically different from those that reached incubation in terms of observed skew during laying (Mann-Whitney $U=76.00$, $n_1=9$, $n_2=13$; $P=0.24$), approximately 56% had high skew values, equal to or greater than 0.50 (mean= 0.52 ± 0.36). In contrast, only 31% of successful nests had a reproductive skew during laying above 0.50 (mean= 0.34 ± 0.30).

For an overall population index of observed reproductive skew we discarded nesting bouts where there was only a single female laying eggs, since these clutches were almost always doomed to failure, and usually comprised an incomplete clutch of only 1–2 eggs. For each nesting bout (Table 2) we show the simulated reproductive skew value with random assignment of eggs to all potentially breeding females in the group. These values were calculated both for the laying phase (prior to clutch reduction through egg ejection) and also for the incubation phase. In this latter phase, calculation was not possible in cases where ≤ 1 egg survived the ejection phase (e.g. Table 2, 1999/Evani bout no. 2). Observed skew did not differ statistically from random skew values, considering all bouts during the laying phase: observed skew was lower than random in 13 of 19 (68%) nesting bouts (two-tailed sign test, $P=0.17$). For the incubation phase this was also the case, with observed skew lower than random in 7 of 11 (64%) nests (two-tailed sign test, $P=0.55$). Taking all bouts together, we calculated means for observed and random skew for laying (observed skew mean= 0.42 ± 0.27 , $n=11$; random skew mean= 0.39 ± 0.12 , $n=19$) and incubation (observed skew mean= 0.36 ± 0.26 , $n=21$; random skew mean= 0.51 ± 0.15 , $n=11$). We found no differences between the mean observed reproductive skew and random skew during either nesting phase (laying: Mann-Whitney $U=138.50$, $n_1=20$, $n_2=19$; $P=0.15$; incubation: Mann-Whitney $U=56.00$, $n_1=11$, $n_2=11$; $P=0.79$).

Discussion

Previous studies of guira cuckoos showed low levels of relatedness among co-breeders and a lack of ecological constraints (Quinn et al. 1994; Macedo and Bianchi 1997). Under these conditions, classical ‘concessions’ models predict a low level of reproductive skew, while

the more recent ‘beneficial sharing’ model predicts that for taxa where young are costly to produce, reproductive skew should be high (Reeve and Ratnieks 1993; Cant 1998; Clutton-Brock 1998; Reeve et al. 1998; Cant and Johnstone 1999).

Available genetic data are too limited yet to establish whether low relatedness is a general phenomenon in the species or whether there is considerable variability in kinship levels among groups. This aspect needs further research, especially to determine whether variability in kinship in different groups is directly associated with fluctuating levels of skew.

The study revealed varying degrees of skew among groups and within groups across repeated nesting bouts. While average reproductive skew for all groups in the laying and incubation phases is moderate, it varies quite widely in a case-by-case analysis. First nesting bouts in the season had a higher average reproductive skew than later attempts, but this difference was not significant. These first bouts, where one or a few females monopolized reproduction, generally resulted in total nest failure before a complete clutch was laid, due to ejection of eggs and nest desertion. Thus, it appears that in this species, behavioral mechanisms operate to avoid complete monopoly of reproductive opportunities within groups. Females, whatever their status, are apparently able to eject each other’s eggs and achieve some breeding success. As the rainy season advances and chances for additional nesting bouts decrease, it is possible that a more equitable distribution of reproductive opportunities among group members during the egg-laying phase contributes toward nesting success. This is a similar situation to that of the acorn woodpecker (*Melanerpes formicivorus*), a polygynandrous breeder that also nests jointly, and where females share reproduction more equitably than expected by skew models, probably due to their capacity to eject eggs and the inability to defend eggs from other group members (Haydock and Koenig 2002).

Despite the low kinship that has been previously found within guira cuckoo groups, the observed skew indices calculated for individual nesting bouts were not always low, as predicted by ‘concessions’ models. With our data we cannot distinguish between the ‘beneficial sharing’ model (Cant and Johnstone 1999) and the ‘incomplete control’ model as potential explanations for the skew patterns we observed. For that we would need some behavioral measure of the competitive ability among females. But clearly, the results point in the direction of: (1) retention of female co-breeders within groups, even with ample dispersal opportunities; (2) general low levels of relatedness within groups, but with some variability; and (3) some equitability in the distribution of reproductive opportunities.

In considering the population as a whole, we found in both nesting and incubation phases that approximately 35% of bouts had observed skew values above the random simulated skew compared to 65% with skew values below, a non-significant deviation. Additionally, the mean observed skew for the population did not differ

from random skew for either nesting phase. These results suggest that reproductive dominance is not characterized in any simple way.

Reproductive partitioning patterns of guira cuckoos elucidated in this study show little similarity to what has been proposed for the communally nesting anis. Vehrencamp (1977) indicated that last-laying 'alpha females' of groove-billed anis postpone egg-laying while earlier-laying females have their individual clutches reduced through egg ejection by those in the group that have not initiated egg laying. This hypothesis was based upon the identification of individual female eggs through visual assessment of egg morphology. The 'alpha female', as designated in that model, was the most successful due to a skew in incubated eggs and fledged young. This conclusion was based on the following assumptions (Vehrencamp 1977):

1. Females do not recognize their own eggs within a mixed communal clutch.
2. Early-laying females, though producing more eggs, have a lower reproductive success than females laying later, due to egg ejection.
3. Once all females in a group are laying, egg ejection ceases.
4. Last-laying females do not lose any eggs and have a higher reproductive success.
5. Females retain their order of entrance in the laying sequence in repeated nesting bouts of the group.
6. All females participate in all laying bouts of the group.

Most of our results are incompatible with the above premises. We found that egg ownership favored early-laying females, and not last-laying ones, as reported for anis by Vehrencamp (1977) and Loflin (1983). Early-laying guira cuckoo females, like the anis, laid more eggs than late-laying females. However, the egg ejection pressure remained constant, affecting all females independent of their position in entering the laying sequence; different from what was reported for anis. In fact, last-laying females in our study lost over 50% of the eggs they laid. This resulted in more incubated eggs for early-laying females. Additionally, our study showed that females neither retained the order of entering laying, nor all contributed eggs in repeated nesting bouts of their groups. While we do not know whether female guira cuckoos can recognize their own eggs, in the ostrich (*Struthio camelus*) females preferentially include their own eggs in the incubated clutch while ejecting eggs of others (Bertram 1979; Kimwele and Graves 2003).

There are two arguments to explain the differences between guira cuckoos and anis. First, there may be subtle yet important differences between their social systems, leading to reproductive strategies and patterns in anis that diverge from those of the guira cuckoo. Ani groups have only up to four or five females (Vehrencamp 1977; Loflin 1983), whereas guira cuckoo groups may contain up to 10 reproductive females. Anis may have a higher coefficient of relatedness within groups, which would predict less

equitable sharing of reproduction. Ecological constraints for anis (e.g., habitat saturation; Koford et al. 1986) do not apply to guira cuckoos, suggesting that the selective factors for breeding in groups may be different. It would be of interest to investigate whether increasing levels of complexity in terms of group size, number of females, and nesting attempts per season in the crotophagine species (*Crotophaga sulcirostris*, *C. ani*, *C. major* and *G. guira*) lead to more equitable reproductive partitioning, taking into consideration degree of relatedness among co-breeders and the presence/absence of ecological constraints.

A second argument that could explain the divergence found in the reproductive patterns of these species is that the methodology used by Vehrencamp (1977) and Loflin (1983) to determine female egg ownership produced a lower resolution of egg differences. At the time of their studies, molecular biochemical techniques were not readily available, and egg morphometry (length, width, weight) was applied to distinguish individual female eggs within the communal clutch as well as the numbers of eggs laid and ejected to estimate the number of laying females. For many species, egg characteristics such as size, shape, color and eggshell patterns are less variable within the clutch of individual females than among clutches of different individuals (Kendeigh et al. 1956; Baerends and Hogan-Warburg 1982; Ricklefs 1984; Croxall et al. 1992; Reed and Oring 1997). While egg morphometry was of critical importance in demonstrating joint nesting in anis and guira cuckoos, more modern techniques like genetic fingerprinting and use of egg protein profiles may be necessary to resolve reproductive skew, individual strategies, group membership and, ultimately, the fitness consequences of reproductive sharing.

The large number of females in guira cuckoo groups may lead to a more egalitarian reproductive partitioning during egg laying than has been previously suggested for crotophagines; it may be impossible for a female to maintain a dominant position through an entire reproductive season. Thus, as in acorn woodpeckers (Haydock and Koenig 2002, 2003), behaviorally dominant guira cuckoo females may not be able to either control reproductive partitioning within the group nor grant reproductive concessions to subordinates.

In our study we found that the last-laying female laid fewer eggs, which is comparable to what has been found for anis. These eggs are often laid toward the very end of the laying period, sometimes even after incubation has started. But contrary to anis, these eggs are frequently ejected. Possibly, egg ejection is carried out not only by females participating in the current nesting bout, but also by group females not involved in that nesting attempt, and which do not risk losing any reproductive investment of their own.

The apparent flexible female reproductive hierarchy in guira cuckoo groups may explain why individuals join and remain in groups despite a high degree of conflict, involving both egg and chick losses. In a rigid hierarchy,

a subordinate female facing continuous and consistent ejection of her eggs might find it more profitable to leave the group and breed independently, especially in an unsaturated habitat. Since joint nests are the most common breeding option in guira cuckoos and anis, presumably there are net reproductive and/or survival advantages for all group members.

Based on our results, it would be difficult to determine whether or not there is a dominant female in terms of reproductive partitioning. Early-laying females apparently have an advantage, considering the number of eggs that eventually survive to incubation, since they invest heavily in egg production but do not suffer a greater relative loss than later-laying females. However, since females apparently do not retain their position in the laying sequence in repeated nesting bouts of the group, nor do they contribute to all nesting bouts, these early and late positions appear to alternate among females. Additionally, although varying degrees of reproductive skew occur in sequential nesting attempts of a group, these values do not always apply to the same group of individuals.

The position in the laying sequence may be important, but only within a broader context, because the best overall strategy seems to be the frequency with which a female contributes to repeated nesting bouts of the group. Females with the highest proportion of incubated eggs over an entire season were those that contributed to two and even three nesting bouts. The scarcity of females that attempted three nesting bouts in a season, however, may be an indication that this tactic may involve costs and may not be advantageous. Obviously, the tactic of choice may ultimately depend upon the groups' ability to renest frequently.

The communal breeding system of the crotophagines presents a challenge in testing the different models of reproductive skew currently available. For guira cuckoos, reproductive dominance hierarchies among breeders seem to be feebly enforced, resulting from a combination of factors. There appears to be a low degree of relatedness among breeders and a lack of ecological constraints, which act in concert with a high number of potentially reproductive females and a very long breeding season, allowing repeated opportunities for nesting. There is mixed maternity within nests and lack of reproductive monopoly, as females apparently can subvert this through egg ejection. Resource abundance may be one key factor to explain the capacity of a female to initiate laying and produce a larger clutch that can suffer high egg losses but still yield a fair measure of reproductive success. An early-laying female may be able to store enough body nutrient reserves to produce a larger clutch simply by not expending energy in feeding young in a previous nesting bout where she was not heavily involved in egg laying, or did not reproduce at all. Additionally, with larger groups there are greater chances of females having more equal competitive abilities, or acquiring them through the extended breeding seasons of tropical regions.

In conclusion, despite the rapid expansion of skew theory and modeling approaches, most of the support for

these models has come from insects in the order Hymenoptera. Our results are not consistent with 'concessions' models, and this may reflect the fact that vertebrate societies exhibit greater variability and behavioral flexibility. Modeling approaches that take into consideration the more complex reproductive strategies of vertebrates would provide broader and more accurate predictions for future studies. The increasing number of sophisticated biochemical and molecular tools should help to resolve previously unknown kinship levels among individuals, perhaps the most important variable in the study of reproductive skew.

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